

# Comparison of calculated and measured foliar O<sub>3</sub> flux in crop and forest species

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*Using a new system to concurrently measure H<sub>2</sub>O, O<sub>3</sub>, and CO<sub>2</sub> flux, the conventional method of calculating O<sub>3</sub> flux generally overestimated direct measures by 25–50%.*

## Abstract

We designed a new gas exchange system that concurrently measures foliar H<sub>2</sub>O, O<sub>3</sub>, and CO<sub>2</sub> flux (HOC flux system) while delivering known O<sub>3</sub> concentrations. Stomatal responses of three species were tested: snapbean, and seedlings of California black oak (deciduous broadleaf) and blue oak (evergreen broadleaf). Acute O<sub>3</sub> exposure (120–250 ppb over an hour) was applied under moderate light and low vapor pressure deficits during near steady state conditions. The rate of stomatal closure was measured when the whole plant was placed in the dark. An adjacent leaf on each plant was also concurrently measured in an O<sub>3</sub>-free cuvette. Under some conditions, direct measurements and calculated foliar O<sub>3</sub> flux were within the same order of magnitude; however, endogenously low gs or O<sub>3</sub> exposure-induced depression of gs resulted in an overestimation of calculated O<sub>3</sub> fluxes compared with measured O<sub>3</sub> fluxes. Sluggish stomata in response to light extinction with concurrent O<sub>3</sub> exposure, and incomplete stomatal closure likewise underestimated measured O<sub>3</sub> flux.

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## 1. Introduction

In the level II approach of the critical levels concept of the UN-ECE (Fuhrer et al., 1997), a flux-based measurement rather than a foliar O<sub>3</sub> exposure metric has been proposed to set regulatory limits for ambient O<sub>3</sub> concentrations. Leaf-level O<sub>3</sub> flux is calculated using leaf conductance (gs) and hourly O<sub>3</sub> concentration, with a multiplying constant to accommodate the difference in diffusivity between water vapor and O<sub>3</sub>. O<sub>3</sub> flux, expressed on a leaf surface area or an individual stoma value, is the regulatory entity. For flux calculation, the bulk air O<sub>3</sub> concentration is used as that concentration next to the

stomata, assuming that the internal O<sub>3</sub> concentration is zero. This approach ignores the leaf boundary layer, and the possibility that O<sub>3</sub> breaks down within the boundary layer.

The question of which leaf conductance to use in the calculation is debatable: gs measured before or after exposure, an average over the day, or an hourly gs value multiplied by an hourly average O<sub>3</sub> concentration in the air could be used. In fact, the Emberson et al. (2000) model uses maximum assimilation (A) data, and the idealized relationship between A and gs (Farquhar and Sharkey, 1982) to estimate maximum gs. In the model, this constructed gs is modified by light levels, temperature, vapor pressure deficit, soil moisture deficit, and phenology, which is then used to calculate flux (as the regulatory entity) using hourly average O<sub>3</sub> concentrations. Although the model helps establish regions at risk, our concern is that known effects of O<sub>3</sub> exposure on stomatal behavior will result

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in significant error in the regulatory entity, and the predictive capability of the model with increases in global O<sub>3</sub> concentrations (Hough and Derwent, 1990) will deteriorate through time.

Ozone exposure modifies stomatal conductance. Aberrant stomatal behavior with O<sub>3</sub> exposure was first reported two decades ago, when both Reich and Lassoie (1984 for poplar, *Populus deltoides* × *trichocarpa*) and Keller and Häslar (1984 for Norway spruce, *Picea abies* (L.) Karst, and white fir, *Abies alba* Miller) described ‘sluggish’ stomatal responses to changes in light. Several authors have continued to report on this response as well as O<sub>3</sub>-induced sluggish response to changes in vapor pressure deficits (sugar maple, *Acer saccharum* Marsh, Tjoelker et al., 1995; Scots pine, *Pinus sylvestris* L., Kellomäki and Wang, 1997). Stomatal aberrations can last up to 10 days after chronic O<sub>3</sub> exposure has ceased (Paoletti, 2005).

Sluggish stomatal responses with O<sub>3</sub> exposure suggests an uncoupling of the normally tight relationship between A and gs (Paoletti and Grulke, 2005) that is assumed in most physiologically based modeling. Sluggish stomata are probably very slowly coming to (theoretical) equilibrium with A under steady-state conditions. However, such ‘steady state’ conditions are not common in the field and functionally, A cannot be considered to be tightly coupled with gs with chronic O<sub>3</sub> exposure or acute events. Incomplete stomatal closure can occur with moderate and above O<sub>3</sub> exposures (Wieser and Havranek, 1995; Matyssek et al., 1995), and can have a significant effect on total O<sub>3</sub> flux into the leaf. If the stomatal response is altered by exposure to O<sub>3</sub>, then there may be large discrepancies in the regulatory entity as calculated, due to stomatal uptake of O<sub>3</sub>.

There have been only three publications that report direct measurements of foliar O<sub>3</sub> flux (Laisk et al., 1989; Moldau and Bichele, 2002; Moldau et al., 1990). In their experiments, the high flow requirement of commercially available O<sub>3</sub> monitors was skirted by injecting the lower flow, exiting sample air into a higher flow ‘carrier’ gas to the O<sub>3</sub> monitor, much like one would use a gas chromatograph. In order to accurately detect the diluted sample air by the high flow O<sub>3</sub> monitor, a high O<sub>3</sub> concentration is required (0.2–2.0 ppm). In other publications, known O<sub>3</sub> concentrations have been supplied to the leaf cuvette, but the O<sub>3</sub> concentration of the exiting cuvette air was not measured (Pasqualini et al., 2002; Grulke and Paoletti, 2005): flux was only calculated under concurrent O<sub>3</sub> exposure, and not measured.

Commercial gas exchange systems adsorb almost all of O<sub>3</sub> supplied directly to the cuvette, and the majority of conductance measurements reported in the literature have not been measured concurrently with O<sub>3</sub> exposure. We designed and demonstrate here the use of a new gas exchange system that delivers known O<sub>3</sub> concentrations to leaves and concurrently measures foliar H<sub>2</sub>O, O<sub>3</sub>, and CO<sub>2</sub> flux (HOC flux system). Two custom, low-flow, fast-response O<sub>3</sub> monitors were designed for the purpose of maintaining O<sub>3</sub> at biologically realistic moderate (50 ppb) to acute (200+ ppb) O<sub>3</sub> levels in a leaf cuvette. The HOC system allows a comparison of calculated

and directly measured foliar O<sub>3</sub> flux. We have conducted measurements with concurrent O<sub>3</sub> exposure for three species: snapbean, California black oak, and blue oak—as a proof-of-concept. In this paper, the HOC system performance was evaluated, and stomatal behavior response to changes in light level with and without concurrent O<sub>3</sub> exposure are presented. The HOC system was used to elucidate some of conditions under which calculated O<sub>3</sub> flux (the regulatory entity) may not be representative, and differ from that of direct measures of foliar uptake.

## 2. Materials and methods

### 2.1. Choice of species

Three species in different physiognomic classes were chosen for flux measurements: an annual crop (an O<sub>3</sub>-sensitive and an O<sub>3</sub>-insensitive variety of snapbean, *Phaseolus vulgaris*), broadleaf deciduous tree seedlings (California black oak, *Quercus kelloggii*), and broadleaf evergreen tree seedlings (blue oak, *Quercus douglasii*). The snapbean O<sub>3</sub>-sensitivity types were developed at the Raleigh USDA-ARS by Drs. Fitz Booker, Ed Fiscus, and Kent Burkey. In their studies, the sensitive phenotype had greater gs in low O<sub>3</sub> levels, but lower total plant water use due to lower total leaf area. The insensitive phenotype had greater A and gs when exposed chronically to moderate O<sub>3</sub> levels (70 ppb), and higher total plant water use (E.L. Fiscus, pers. commun.).

California black oak is a drought-adapted species common throughout California in the transition between chaparral and the montane, mixed conifer forest (McDonald, 1990a). California black oak was symptomatic in southern California during the high O<sub>3</sub> exposures of the mid 1970s (Miller et al., 1980), but foliar injury under ambient field conditions has not been reported recently. Earlier onset of senescence and stomatal aberrations with elevated nitrogen deposition and high background O<sub>3</sub> exposure has been reported in this species in the field (Grulke et al., 2005). Blue oak is found in the dry, interior foothills throughout California (McDonald, 1990b), but little physiological data are available for this species.

### 2.2. Plant propagation and experimental design

All three species were grown in a temperature- and humidity-controlled greenhouse in Riverside, California. Gas exchange measurements were conducted on snapbean that were of an age between six to nine weeks from time of germination in a greenhouse. Direct measures of foliar O<sub>3</sub> flux were conducted on four O<sub>3</sub>-sensitive, and four O<sub>3</sub>-insensitive snapbean plants. Oaks were germinated from acorns collected locally, and grown in greenhouses until 3–5 years old. In mid-March, 2005, plants were placed in open-top exposure chambers and subjected to a chronic O<sub>3</sub> exposure of 70 ppb for 8 h per day for 1 (California black oak) or 2 months (blue oak). One plant of each oak species were placed three charcoal-filtered or three elevated open top chambers.

In the greenhouse, paired measurements of gas exchange with and without cuvette O<sub>3</sub> were conducted on adjacent leaves on each snapbean and oak seedling. The O<sub>3</sub>-free leaf gas exchange measurements were made with a LiCor Instr. Model 6400 open system (Lincoln, NE), referred to here as the HC system (only H<sub>2</sub>O and CO<sub>2</sub> flux). The leaf gas exchange measurements with elevated O<sub>3</sub> were made with the HOC system described below, which used two custom O<sub>3</sub> monitors (one for the reference air and one for the sample air) for the O<sub>3</sub> flux, and a second LiCor, Instr. Model 6400 (or 6262) for the H<sub>2</sub>O and CO<sub>2</sub> flux from the reference and sample gases (respectively) for the HOC system.

### 2.3. HOC system description

A custom-designed gas exchange system was used for this study because of the inability to experimentally maintain elevated O<sub>3</sub> in the cuvette of conventional gas exchange systems. A prototype of this system that supplied

Fig. 1. Schematic of H<sub>2</sub>O, O<sub>3</sub>, CO<sub>2</sub> (HOC) flux system. Air was fully conditioned and flow controlled (MFC) prior to entering the cuvette. Two Licor model 6400s were used in the system: one for measuring leaf conductance in O<sub>3</sub>-free air (HC system), and one for measuring the reference (REF) and sample (SAMP) air in the HOC system cuvette. The reference and sample air was first passed to a custom low flow, fast response O<sub>3</sub> monitor (O<sub>3</sub> MON), then the flow was read (MFR), and the air was passed to either the reference or to the sample CO<sub>2</sub> and H<sub>2</sub>O infra red gas analyzer (IRGA).

rates at equilibrium. Although  $O_3$  flux in the null cuvette was correlated to cuvette relative humidity (greater  $O_3$  adsorption and/or degradation with higher cuvette relative humidity), measured foliar  $O_3$  flux was not correlated to cuvette relative humidity because null cuvette gas exchange ( $H_2O$ ,  $CO_2$ , and  $O_3$ ) was subtracted from foliar gas exchange rates. Therefore, the null cuvette measures were an effective correction.

Leaf and cuvette air temperature, light level, and signals from the  $O_3$  monitors (and LiCor model 6262 IRGA when in use) were monitored with a data logger (model 21x, Campbell Inc., Logan, UT), and graphed continuously on a laptop screen. Temperatures in the cuvette with illumination ranged from 22 to 27 °C (but changed slowly over the day). Light level was constant during measurements, but varied between 800 and 900  $\mu mol\ m^{-2}\ s^{-1}$  ( $Q$ ) between plants. Relative humidity of the air stream ranged from 22 to 60%, and changed little (2%) for plants with low gs, but changed more (5%) with higher leaf area and higher gs over the course of the illuminated measurements.

Data were merged into a single Excel spreadsheet (from the control leaf with no  $O_3$  exposure in the HC system, the HOC system leaf with acute, short-term  $O_3$  exposure, and the data logger). To calculate  $O_3$  flux, gs was multiplied by  $O_3$  concentration and the constant 0.612 to account for the differences in diffusivity between water and  $O_3$ . The internal foliar  $O_3$  concentration was assumed to be zero (Laisk et al., 1989). Comparable to the regulatory entity, foliar  $O_3$  uptake in  $O_3$ -free air was calculated from HC system gs, and the  $O_3$  concentration of the reference air supplied to the HOC cuvette (HC\_flux(E); see Table 1 for a list of terms). In our experiments, we reported two calculated  $O_3$  fluxes and one measured  $O_3$  flux from the HOC system. The former  $O_3$  fluxes were calculated from gs measured with concurrent  $O_3$  exposure using (1)  $O_3$  concentration of the entering air (HOC\_flux(E)), and (2) the average  $O_3$  concentration in the cuvette ((reference air + sample air)/2, HOC\_flux(A)). Direct measure of foliar  $O_3$  flux was also determined (HOC\_flux(M)) from the difference between the reference and sample air  $O_3$  concentration, the molar flow rate, and the leaf area. If cuticular  $O_3$  uptake were small, the HOC\_flux(M) was expected to be most comparable to HOC\_flux(A). Foliar  $O_3$  flux was reported on a one leaf surface area basis: the majority of the stomata are on the bottom leaf surface in all three species.

### 3. Results

#### 3.1. Comparison of calculated and measured $O_3$ flux

Although measured and calculated  $O_3$  fluxes were within the same order of magnitude, the  $O_3$  flux measured in the HOC system (HOC\_flux(M); see Table 1 for definition of

terms) was much lower ( $5\ nmol\ m^{-2}\ s^{-1}$ ) than the regulatory entity ( $13\ nmol\ m^{-2}\ s^{-1}$ , the calculated  $O_3$  flux using the gs from the  $O_3$ -free HC cuvette (HC\_flux(E)) (Fig. 2). As expected, calculated  $O_3$  flux using the average  $O_3$  concentration in the HOC cuvette (HOC\_flux(A)) was the most similar to measured  $O_3$  flux (HOC\_flux(M)). Calculated  $O_3$  flux using entering air (HOC\_flux(E)) and average cuvette  $O_3$

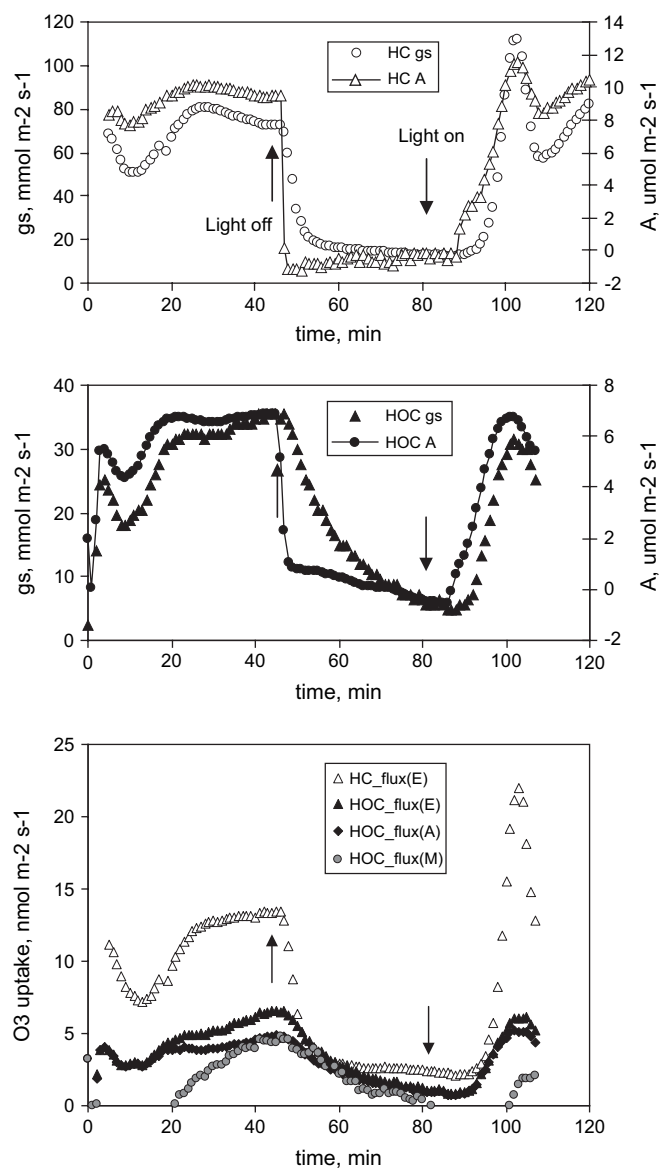


Fig. 2. Response of an  $O_3$ -insensitive snapbean. Stomatal conductance (gs) and assimilation (A) measured in the HC system (top) and in the HOC system (middle). Calculated and measured foliar  $O_3$  fluxes are given in the bottom graph.  $O_3$  flux in  $O_3$ -free air (HC\_flux(E)) was calculated using  $O_3$  concentration of air entering the HOC cuvette.  $O_3$  flux with concurrent  $O_3$  exposure was calculated using the  $O_3$  concentration of air entering the HOC cuvette (HOC\_flux(E)) and the average  $O_3$  concentration in the cuvette (HOC\_flux(A)). Direct measures of  $O_3$  flux (HOC\_flux(M)) were determined from the difference between entering and exiting  $O_3$  concentration in the HOC cuvette, the molar flow rate, and the leaf area in the cuvette. Delay in stabilization of HOC\_flux(M) was due to pressure changes in the sample  $O_3$  monitor when the cuvette was opened to insert the leaf, then closed. Arrows indicate when the external light source was turned off, or on. Terms are defined in Table 1.

Table 1  
Definition of terms

gs	Stomatal conductance in $mmol\ H_2O\ m^{-2}\ s^{-1}$
A	Net assimilation in $\mu mol\ CO_2\ m^{-2}\ s^{-1}$
$O_3$ flux	Foliar $O_3$ flux in $nmol\ O_3\ m^{-2}\ s^{-1}$
HC gs, A	Leaf gas exchange in Licor 6400 cuvette without concurrent $O_3$ exposure
HOC gs, A	Leaf gas exchange in HOC cuvette, with concurrent, short-term, acute $O_3$ exposure
HC_flux(E)	Regulatory entity: calculated foliar $O_3$ flux in Licor 6400 cuvette without $O_3$ exposure, using the $O_3$ concentration of the reference air ( $O_3$ entering (E) the HOC system chamber)
HOC_flux(E)	Calculated foliar $O_3$ flux using HOC gs and $O_3$ concentration of the entering (E) air stream
HOC_flux(A)	Calculated foliar $O_3$ flux using HOC gs and the average (A) cuvette $O_3$ concentration
HOC_flux(M)	Direct measure of foliar (+ cuticle) $O_3$ flux using the difference between entering and exiting air $O_3$ concentration, molar flow rate, and leaf area in the HOC cuvette

concentration ( $\text{HOC\_flux(A)}$ ) also differed because of leaf uptake (6 vs. 5  $\text{nmol m}^{-2} \text{s}^{-1}$ ), but the two fluxes changed in tandem. If cuvette  $\text{O}_3$  concentration had not been known (e.g., if only entering or reference  $\text{O}_3$  concentration were known), calculated  $\text{O}_3$  flux would have been overestimated by 20% (see Pasqualini et al., 2002).

In all measures of  $\text{HOC\_flux(M)}$ , there was an apparent gap in data after the null cuvette measure lasting 20–35 min. One might suspect that this was due to a large volume in the chamber. Instead, the lag before positive fluxes appeared was due to pressure changes from opening and closing the HOC cuvette when the leaf was inserted. The pressure change resulted in a rapid increase in the sample, but not the reference  $\text{O}_3$  monitor, and thus a temporary ‘negative’  $\text{O}_3$  flux (only positive  $\text{O}_3$  flux values graphed, Fig. 2). When the light was extinguished (at 50 min), stomatal aperture declined quickly and all measures of  $\text{O}_3$  flux declined at a more or less similar rate. Because  $g_s$  in both the HC and HOC systems did not reach zero, the calculated  $\text{O}_3$  flux remained above zero, but the measured  $\text{O}_3$  flux ultimately became very close to zero (at 75 min). When the light was turned back on (at 80 min), a temperature-induced pressure change again resulted in a negative  $\text{O}_3$  flux, which then began to recover by 100 min but never fully recovered before the experiment was terminated. Such transitory pressure changes also occur in commercial systems with large changes in light level. Aside from this lag period, calculated  $\text{O}_3$  flux based on  $g_s$  with concurrent  $\text{O}_3$  exposure are good, but not perfect, approximations of foliar  $\text{O}_3$  flux for an insensitive snapbean.

Similar comparisons between calculated and measured  $\text{O}_3$  flux were obtained for California black oak grown in an  $\text{O}_3$ -free environment (Fig. 3). Calculated  $\text{O}_3$  flux based on the average cuvette  $\text{O}_3$  concentration was nearly identical to measured  $\text{O}_3$  flux (after equilibration). If only the entering air  $\text{O}_3$  concentration had been known,  $\text{O}_3$  flux would have been overestimated by 25%. If only  $g_s$  of a leaf in an  $\text{O}_3$ -free cuvette were known (HC system), calculated  $\text{O}_3$  flux would have been overestimated by 50% relative to directly measured flux. For a California black oak under chronic  $\text{O}_3$  exposure, the added stress of concurrent acute  $\text{O}_3$  exposure depressed  $g_s$  by 80% (Fig. 4). Measured  $\text{O}_3$  flux was again very similar to that calculated using system  $g_s$  and average cuvette  $\text{O}_3$  concentration. However, when the black oak seedling was placed in the greenhouse and measured with an  $\text{O}_3$ -free cuvette (HC system with the  $\text{O}_3$ -free greenhouse air), there was evidence of a short-term increase in both A (not shown) and  $g_s$ . Calculated  $\text{O}_3$  flux based on the  $\text{O}_3$ -free cuvette (HC system) measurements greatly exceeded that measured in the HOC system.

If cuticular uptake were detectable with this system, then measured  $\text{O}_3$  flux ( $\text{HOC\_flux(M)}$ ) would always be greater than that calculated ( $\text{HOC\_flux(A)}$ ). However, measured  $\text{O}_3$  flux was greater than calculated  $\text{O}_3$  flux in the HOC system with concurrent  $\text{O}_3$  exposure only half the time. The median range of differences was  $\pm 25\%$ . At equilibrium, the differences between calculated system  $\text{O}_3$  flux and measured system  $\text{O}_3$  flux could not be accounted for by either varying the

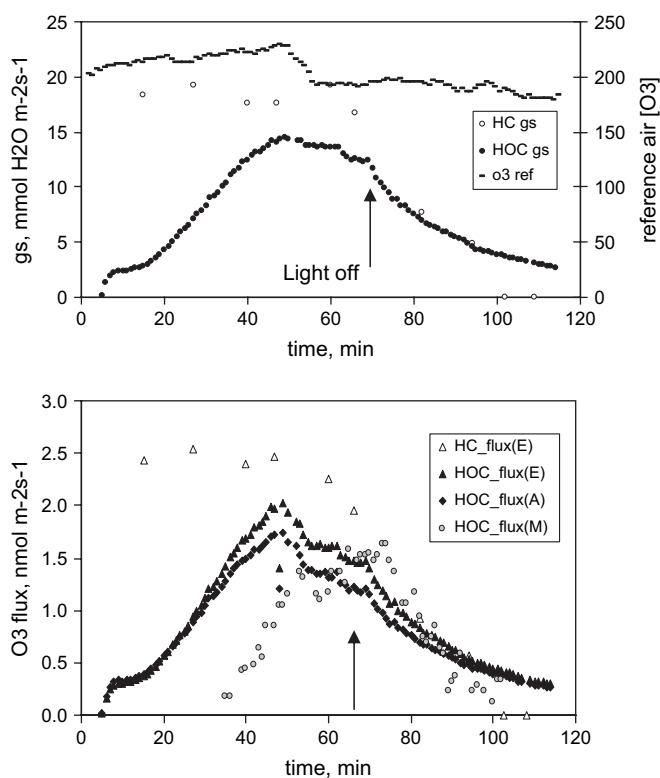


Fig. 3. Response of California black oak grown in  $\text{O}_3$ -free air with and without acute, short-term  $\text{O}_3$  exposure. Time course of reference air  $[\text{O}_3]$ ,  $g_s$  measured in the  $\text{O}_3$ -free cuvette (HC system), and  $g_s$  measured with concurrent  $\text{O}_3$  exposure in the HOC system (top graph). Time course of calculated ( $\text{HC\_flux(E)}$ ,  $\text{HOC\_flux(E)}$ , and  $\text{HOC\_flux(A)}$ ) and measured foliar  $\text{O}_3$  flux ( $\text{HOC\_flux(M)}$ ) given in the bottom graph. Arrows indicate when the external light source was turned off.

cuvette relative humidity (22–60%) or  $\text{O}_3$  concentration (0.1–0.25 ppm). However, there was a species difference: blue oak had, on average, 30% lower measured  $\text{O}_3$  flux than calculated flux. The leaves of this species are hairy, and measured  $\text{O}_3$  flux would have been expected to be greater, not lower than that calculated. In California black oak, measured and calculated  $\text{O}_3$  flux were within 2% on average, and measured  $\text{O}_3$  flux was slightly greater than that calculated. In snapbean, measured  $\text{O}_3$  flux was 40% greater than calculated  $\text{O}_3$  flux based on average  $\text{O}_3$  concentration in the cuvette. There were no differences between sensitive or insensitive phenotypes.

$\text{O}_3$  flux based on reference air  $\text{O}_3$  concentration ( $\text{HC\_flux(E)}$ ) was always greater than  $\text{HOC\_flux(E)}$ . The ratio of HC to HOC  $\text{O}_3$  flux (E) was significantly correlated to the ratio of lf to sys  $g_s$  ( $r = 0.76$ ), but less correlated to cuvette relative humidity ( $r = 0.43$ ) and reference  $\text{O}_3$  concentration ( $r = -0.30$ : the higher the  $\text{O}_3$  concentration, the lower the  $g_s$  and  $\text{O}_3$  flux in the HOC system).

### 3.2. Stomatal aberrations with direct $\text{O}_3$ exposure

In snapbean, approximately one hour of acute  $\text{O}_3$  exposure decreased maximum  $g_s$  and A under moderate light (Table 2). Maximum  $g_s$  was depressed by 50% in the  $\text{O}_3$ -sensitive

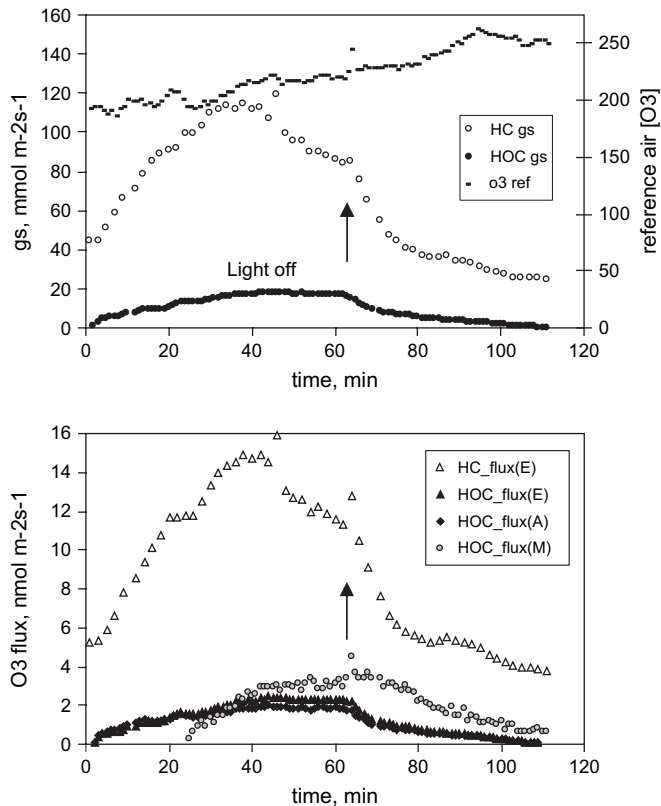


Fig. 4. Response of California black oak grown in chronic, month long  $O_3$  exposure in OTCs, then exposed to either  $O_3$ -free air or acute  $O_3$  exposure. See Fig. 3 for further description.

phenotype and by 60% in the  $O_3$ -insensitive phenotype. Maximum gs in blue oak was similarly depressed by short-term, acute  $O_3$  exposure in both activated-charcoal filtered OTCs, but differences were not significant. The maximum A at equilibrium followed the same patterns in blue oak and snapbean: short-term acute  $O_3$  exposure depressed A significantly in both species. Because California black oak seedlings exposed to chronic  $O_3$  exposure rapidly increased gas exchange when transferred to the greenhouse (see Fig. 4), there were no significant effects of chronic or acute  $O_3$  exposure on either A or gs, or the rate of stomatal closure in this study.

When the cuvette (and whole plant) was placed in the dark, stomatal closure was sluggish when concurrently exposed to  $O_3$  (HOC system) relative to gs measured in  $O_3$ -free air (HC system). For insensitive snapbean (Fig. 2), reduction in stomatal aperture with light extinction was twice as slow with acute  $O_3$  exposure as in  $O_3$ -free air. Stomatal response to increasing light was slightly different in the HC and HOC systems, but this difference did not wholly account for sluggish response with concurrent  $O_3$  exposure. For blue oak, acute  $O_3$  exposure significantly reduced the rate of stomatal closure in response to extinguishing the light, whether the seedlings were grown in charcoal-filtered air or elevated  $O_3$ . California black oak had a similar pattern, but differences were not significant.  $O_3$ -sensitive snapbean had the fastest stomatal closure in an  $O_3$ -free cuvette when the plant was placed in the dark.

Table 2

Summary of maximum gs and A under moderate light at equilibrium with and without concurrent, short-term, acute  $O_3$  exposure, and rate of stomatal closure (change in gs per min) in response to the whole plant being placed in the dark

Species	Chronic	Acute	Max gs	gs reduction rate	Max A
QD	C	C	121 (47)	2.89 (0.65)a	7.68 (0.48)a
QD	C	$O_3$	50 (15)	0.84 (0.20)b	3.85 (0.46)b
QD	$O_3$	C	104 (25)	2.47 (0.25)a	9.28 (0.32)a
QD	$O_3$	$O_3$	57 (18)	1.14 (0.31)b	4.81 (0.71)b
<i>p</i>			—	0.008	<0.001
QK	C	C	34 (10)	1.06 (0.39)	3.69 (0.99)
QK	C	$O_3$	34 (13)	0.64 (0.41)	3.57 (1.99)
QK	$O_3$	C	69 (9)	3.56 (2.68)	4.62 (1.4)
QK	$O_3$	$O_3$	30 (7)	0.29 (0.04)	2.10 (0.79)
<i>p</i>			—	—	—
PV	Sensitive	C	96 (14)a	4.82 (1.61)a	12.04 (0.86)a
PV	Sensitive	$O_3$	45 (6)b	1.00 (0.25)b	7.13 (0.63)b
PV	Insensitive	C	104 (19)a	3.11 (0.24)c	11.17 (1.01)a
PV	Insensitive	$O_3$	34 (4)b	0.74 (0.04)b	6.44 (0.97)b
<i>p</i>			0.019	<0.001	0.003

Plants were exposed to activated charcoal filtered air or chronic  $O_3$  exposure in open top chambers for 1 month (QK, *Quercus kelloggii*) or 2 months (QD, *Quercus douglasii*). Snapbean (PV, *Phaseolus vulgaris*) was grown in a filtered greenhouse and had only short-term acute  $O_3$  exposure. The significance (*p*) is reported only where significant (<0.05) for a 1-way ANOVA (SPSS 2000), treating each combination as a separate entity. Numbers in parentheses are mean  $\pm$  1 S.E.

In two California black oaks, one chronically exposed to  $O_3$  and one grown in activated charcoal filtered air, gs after 40 min in the dark was  $>30 \text{ mmol m}^{-2} \text{ s}^{-1}$  when measured concurrently with acute  $O_3$  exposure. Gs measured on an adjacent leaf on the same plant, in the dark, in an  $O_3$ -free cuvette, was undetectable. In two blue oaks, also one chronically exposed to  $O_3$  and one grown in activated charcoal filtered air, gs measured concurrently with acute  $O_3$  exposure was 30% greater than that measured in an  $O_3$ -free cuvette. In these plants, calculated  $O_3$  flux based on the HC system gs (the regulatory entity) greatly underestimated flux.

Several  $O_3$  metrics were tested for the significance of correlation with maximum gs and A, and rate of stomatal closure (Table 3), including the average  $O_3$  concentration, dose,  $O_3$ -flux(A), and  $O_3$ -flux(M). For California black oak, no correlation coefficient was significant. For snapbean, all  $O_3$  metrics tested had a significant, negative correlation with maximum gs and A, and the rate of stomatal closure. For blue oak, the average  $O_3$  concentration had the highest correlation coefficient with maximum gs and A, and stomatal closure. Dose,  $O_3$ -flux(A), and  $O_3$ -flux(M) were not correlated with maximum gs, but were significantly correlated to rate of stomatal closure and maximum A. The average  $O_3$  concentration had the highest correlation coefficient.

#### 4. Discussion

Calculated foliar  $O_3$  flux from leaves measured in  $O_3$ -free cuvettes (HC\_flux(E), the regulatory entity) overestimated measured foliar  $O_3$  flux with concurrent  $O_3$  exposure

Table 3  
Physiological response to acute O<sub>3</sub> exposure

Species	Max gs	Stomatal closure rate	Max A
QD			
Ave [O <sub>3</sub> ]	−0.599	−0.807	−0.915
Dose	—	−0.724	−0.795
O <sub>3</sub> flux (A)	—	−0.610	−0.567
O <sub>3</sub> flux (M)	—	−0.699	−0.784
PV			
Ave [O <sub>3</sub> ]	−0.693	−0.836	−0.838
Dose	−0.631	−0.775	−0.804
O <sub>3</sub> flux (A)	−0.564	−0.702	−0.760
O <sub>3</sub> flux (M)	−0.646	−0.729	−0.772

Physiological response (maximum gas exchange at equilibrium in moderate light, and rate of change of gs to extinguishing the light) to acute O<sub>3</sub> exposure in a HOC cuvette was tested for correlation with average O<sub>3</sub> concentration in the cuvette (ave [O<sub>3</sub>]), dose (ppb h), calculated foliar flux (HOC\_flux(A)), and measured foliar flux (HOC\_flux(M)). See Table 2 for acronyms. The sign and correlation coefficient (*r*) is given only for significant values (*p* < 0.05) (SPPlus 2000). There were no significant relationships for California black oak.

(HOC\_flux(M)). Reductions in gs as a result of concurrent O<sub>3</sub> exposure further reduced gs and measured foliar O<sub>3</sub> flux, and resulted in greater overestimation by the regulatory entity. Sluggish stomatal responses to changes in environmental conditions (demonstrated here for light), or incomplete stomatal closure, resulted in underestimation by the regulatory entity relative to direct measurements. In a few plants of both California black oak and blue oak, there was a lack of stomatal closure when the plant was placed in the dark. Under these conditions, the regulatory entity underestimated direct measures of foliar O<sub>3</sub> flux by 80%. Concurrent short-term, acute O<sub>3</sub> exposure depressed calculated O<sub>3</sub> flux (HOC\_flux(E)) by >20% relative to calculated O<sub>3</sub> flux for foliage in an O<sub>3</sub>-free cuvette (HC\_flux(E)). Under concurrent acute O<sub>3</sub> exposure, calculated O<sub>3</sub> flux using reference air O<sub>3</sub> concentration (E) was 15% greater than calculated O<sub>3</sub> flux using the average cuvette O<sub>3</sub> concentration (A). Calculated O<sub>3</sub> flux using the average cuvette O<sub>3</sub> concentration with concurrent O<sub>3</sub> exposure was very similar to that obtained by direct measurement of foliar O<sub>3</sub> flux ((HOC\_flux(M)). When exposure, dose, and calculated and measured O<sub>3</sub> flux were tested, the average cuvette O<sub>3</sub> concentration had the highest correlation coefficient with maximum gs, A, and rate of stomatal closure (Table 3).

Stomatal conductance and foliar O<sub>3</sub> flux was greatest in snapbean, followed by blue oak, followed by California black oak. Variation in endogenous rates of gs within a species also significantly modified O<sub>3</sub> flux. California black oak and blue oak seedlings chronically exposed to O<sub>3</sub> had relatively low gas exchange, and had little response to short-term, acute O<sub>3</sub> exposure, because gs and O<sub>3</sub> flux into the leaf was also low. In these individuals, acute O<sub>3</sub> exposure did not result in sluggish stomatal closure when placed in the dark (Fig. 4): the rate of stomatal closure was nearly the same in both charcoal-filtered and chronic O<sub>3</sub> exposure.

Besides cuticular adsorption, biogenic hydrocarbons emitted from foliage react with O<sub>3</sub> (Fehsenfeld et al., 1992). *Phaseolus vulgaris* does not emit either isoprene or monoterpenes, the most

common biogenic VOCs (Arey et al., 1991). Black oaks emit 2–3 times more isoprene than blue oak (Geron et al., 2001). Monoterpene emission was not found in blue oak (Tanner and Zielinska, 1994). Our data suggest that non-stomatal flux of O<sub>3</sub> was low in all three species. The best empirical support for lack of significant, non-stomatal flux was that there was little difference in O<sub>3</sub> flux between a null cuvette measurement and leaves in the cuvette in the dark. California black oak had nearly identical measured and calculated O<sub>3</sub> flux (based on the average concentration). In wheat, non-stomatal deposition was small compared to the stomatal uptake (Pleijel et al., 2004).

Models developed (Emberson et al., 2000; Grunhage et al., 2001) to estimate crop or forest species use steady-state parameters. However, even in an open-grown tree, on the southern aspect, light varied >2 standard deviations from the mean in low (30–200 Q), medium (600–900 Q), and high (1400–1800 Q) average background light levels two thirds of the time (Grulke, unpubl. data for mature *Pinus ponderosa*). Despite the errors associated with estimating non-foliar O<sub>3</sub> flux in forest or crop stands, whole ecosystem flux data (Kurpius et al., 2002; Matyssek et al., 2004) is perhaps the best check on modeled O<sub>3</sub> flux because of the concurrent O<sub>3</sub> canopy exposure, and varying environmental conditions.

Despite two decades of research, stomatal aberrations in response to O<sub>3</sub> exposure have been largely ignored. Current modeling efforts predict O<sub>3</sub> effects on plants, but the modeling approach for the EU-ECE level II (Emberson et al., 2000; Grunhage et al., 2001) uses assimilation models (Farquhar and Sharkey, 1982) with calculated gs (and calculated O<sub>3</sub> flux from calculated gs). In this environment, the potential for incorporating future improvements in our understanding of stomatal behavior into the flux-based O<sub>3</sub> metric are limited. The consequence for using an assimilation-derived modeling approach to estimate O<sub>3</sub> flux is to minimize the importance of direct O<sub>3</sub> effects on plant water balance and the lack of predictive capabilities of models to establish the link between O<sub>3</sub> exposure, increased susceptibility to drought stress, and deterioration of forest health.

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